We conclude that the two strands are effectively stapled together through a large number of weak bonds involving T4 ligase. The absence of a similarly strong transformation of DNA in free solution points towards the necessity of parallel pre-alignment through the nanotube. We point to the fact that the formation of hairpins and the 1-d scanning of strands past each other solves the kinetic problem of forming a loop in a maze-like environment.

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The Binding Parameters of Integration Host Factor to the Lambda Phage Cosi1 Site and Holliday Junction DNA

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Integration Host Factor (IHF) is a heterodimeric DNA-binding protein produced by E.coli. IHF interacts with the minor groove and phosphodiester backbone of the DNA to create sharp bends in the DNA and is often referred to as an architectural protein. Although the protein binds sequence-specifically, it also recognizes DNA conformation. Prokaryotic cellular processes involving IHF include viral integration, replication and activation of gene expression.

We have recently shown that IHF exhibits high binding affinity for the Holliday or four-way junction DNA (4WJ), an intermediate in homologous recombination where strands from different chromosomes cross over. Förster Resonance Energy Transfer (FRET), where the 5'-ends of DNA strands are labeled with fluorophores, is used to determine the conformational changes of the 4WJ when interacting with DNA-binding proteins. These measurements have shown that the 4WJ opens upon binding with IHF, which is consistent with data promoting the protein's involvement in recombinase action. Subsequent FRET experiments performed confirm this observation, and provide a value for the efficiency of binding and the degree of distortion induced.

Interactions between IHF and the cos I1 site of DNA from the lambda bacteriophage will also be explored. It is known that IHF induces bending at cos sites. The affinity of binding to Δ cos DNA is compared to that between IHF and its consensus sequence (H1) in E. coli DNA. Gel mobility shift assay (GMSA) and solutions experiments will measure the binding and affinity of IHF with the cos I1 site. FRET experiments with labeled cosI1 DNA will reveal the distortion induced upon IHF binding and these results will be compared to those with IHF binding to the H1 site.

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Structural Insight into Protein Recognition by RNA Aptamers

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RNA aptamers are structured single-stranded oligonucleotides that specifically bind to a broad spectrum of biomolecular targets with as tight as pM affinity. The structural stability and diverse functionality of aptamers have enabled their use as diagnostic tools, inhibitors and potential therapeutic agents. However, a detailed understanding of how aptamers specifically bind to selected regions on the surface of proteins and cells lags significantly behind advances in their applications. Solving a high resolution RNA aptamer structure by crystallographic or spectroscopic methods require minimizing the aptamer into a compact form. We show that aptamers can be effectively minimized for structural analysis using chemical mapping to experimentally define the secondary structure and identify tertiary contacts within the RNA and with the target protein. Ribonuclease and SHAPE mapping were used to determine the correct predicted secondary structure of a high affinity aptamer (Lys1) selected against hen egg white lysozyme (KD ~ 30 nM). A deletion variant, minE, was engineered to delete a long apparently unstructured region. Binding assays indicate comparable affinity of lysozyme against minE, KD ~ 20 nM. The lysozyme-minE complex was solved by x-ray crystallography at 2.0 Å resolution yielding a seventh RNA aptamer-protein structure. Solution hydroxyl-radical footprinting confirms the binding interface observed in the crystal. The minE aptamer interacts with a basic face of lysozyme, which is located opposite from the catalytic residues Asp52 and Glu35. The minE aptamer inhibits the function of lysozyme in vitro. The binding of minE distant from the catalytic site suggests that the aptamer inactivates lysozyme allosterically. The long term goal of this study is to develop a systematic approach to aptamer minimization and use solved structures to probe the mechanisms by which RNA aptamers bind their targets and regulate catalytic activity and/or cellular function.

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Nucleic Acid Binding Kinetics of HIV-1 Nucleocapsid Proteins from Single Molecule DNA Stretching

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The human immunodeficiency virus type 1 (HIV-1) Gag protein is essential for retroviral assembly. During viral maturation, Gag is processed to form matrix (MA), capsid (CA), and nucleocapsid (NC). Mature NCp7 is derived from processing of NCp15 and NCp9. NCp7 functions as a nucleic acid chaperone during retroviral replication, in which it rearranges nucleic acids to facilitate reverse transcription and recombination. In this work, we used single-molecule DNA stretching to probe the interactions of these proteins with DNA. Using this technique, we have previously shown that NCp7 destabilizes DNA with rapid kinetics, yielding almost reversible force-extension curves. NCp7 intercalates into dsDNA to keep the two single strands close together while destabilizing them. Surprisingly, multiple stretch and release cycles of DNA in the presence of NCp7 yields changing force-extension curves on the time scale of tens of minutes. If the NCp7 solution is rinsed from the buffer surrounding the DNA molecule, we find that some fraction of the bound protein does not dissociate. The protein only dissociates completely when competitor DNA is introduced to the solution surrounding the stretched DNA molecule. Thus, NCp7 exhibits binding modes on multiple time scales, including both rapid microscopic and slow macroscopic dissociation rates. To test the origin of this surprising behavior and understand how NC-DNA interactions are regulated, we will also apply these methods to NCp9 and NCp15 interactions with DNA. Further studies will elucidate the kinetics of these protein-DNA interactions. *This work was funded in part by Federal Funds from NCI, NIH under contract HHSN261200800001E (RJG).*

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Controlling Drug Resistance in Fungal Systems by Zinc Cluster Proteins

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Drug resistance is a serious complication for patients with an infection, and unfortunately, bacteria and fungi are quickly rendering many therapeutic drugs ineffective. In Saccharomyces cerevisiae, this mechanism is defined as pleiotropic drug-resistance (PDR). Overexpression of drug efflux pumps, which transport drugs out of the cell, is controlled by two major transcriptional regulators, Pdr1p and Pdr3p. Homologous PDR pathways have been identified in other pathogenic species, such as Canadida albicans. Pdr1p and Pdr3p are zinc cluster transcription factors that recognize a pair of CGG repeats in the promoter region of target PDR genes. Interestingly, these transcriptional regulators tolerate sequence variation within the binding site and recognize a small group of variant sites. Pdr1p and Pdr3p have been shown to selectively regulate PDR genes through canonical and variant binding sites. This is observed in the differential regulation of two PDR genes: PDR5, the prominent ABC transporter and HXT11, a hexose transporter. Pdr1p and Pdr3p recognize a variant binding site in the promoter region of PDR5; however, the transcriptional regulators do not recognize the same variant site in the promoter of HXT11. The regulatory mechanism for how canonical and variant binding sites lead to differential PDR gene expression has yet to be explored. These protein/DNA interactions have been studied using two approaches, binding affinity and specificity studies. Fluorescence anisotropy and surface plasmon resonance were used to determine the individual binding affinities of the DNA-binding domain (DBD) for canonical and variant binding sites, and x-ray crystallography was utilized to resolve the molecular interactions between the DBD complex with canonical and variant binding sites. Differences in the binding motifs and in the DBDs of Pdr1p and Pdr3p must allow for differential regulation, making this a unique system for investigation and a possible drug target.

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Towards Pharmacological Modulation of ETS-Dependent Transcription

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Specific inhibition or activation of enzymes and other proteins with small molecules is a widely exploited approach to modulating cellular pathways. Targeting the expression of these proteins at the level of transcription has remained relatively unexplored, however, and may offer significant biotechnological and therapeutic potential. We are interested in pharmacological modulation of genes controlled by members of the ETS family of transcription factors. ETS proteins are widely distributed among metazoan phyla and regulate a functionally diverse array of genes. As a model system, we have characterized the inhibition of sequence-specific binding of the ETS domain of PU.1 (Spi-1) to a natural high-affinity site by distamycin. Using previously established thermodynamic data on this protein-DNA system, we have refined the experimental conditions for surface-plasmon resonance (SPR) overcome